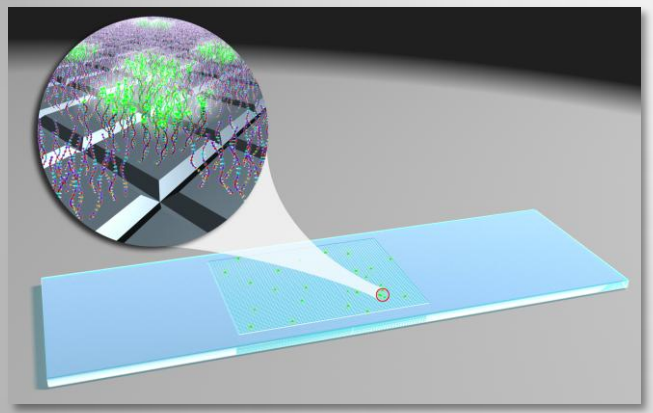
A Sampling of Diagnostic Development at LLNL

Ted Laurence

LLNL-PRES-505692

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under contract DE-AC52-07NA27344. Lawrence Livermore National Security, LLC

Lawrence Livermore Microbial Detection Array (LLMDA) can detect any known virus and bacteria within 24 hours



- 387,156 total probes
- 3,100 viral and bacterial species
- Design involved hundreds of thousands of cluster CPU-hours in DNA probe design and statistical data analysis

Applications

- Broad spectrum pathogen screening
- Identify pathogens associated with cancer
- Diagnose unknown clinical infections from human
- Survey of food pathogen contaminations and trace food pathogen outbreaks
- Discover biomarkers from human diseases and infections

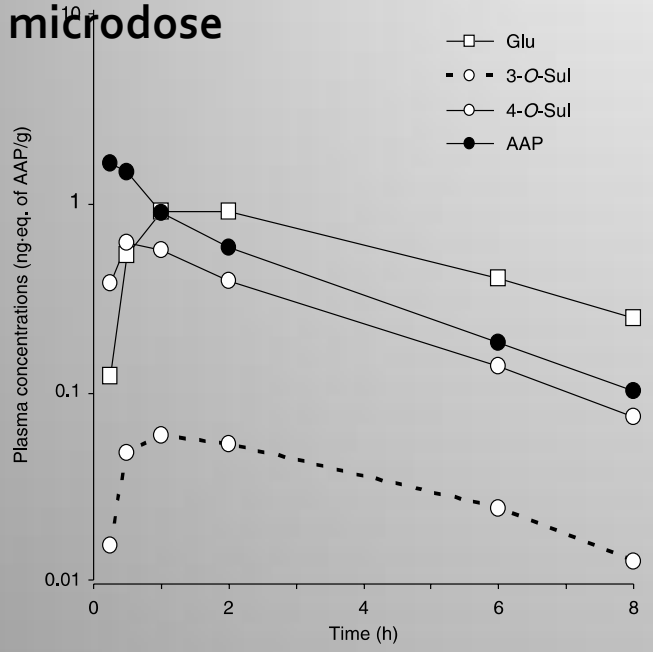
- In 2010, LLMDA identified an adventitious porcine virus from a rotavirus vaccine, a vaccine administered in infants worldwide to prevent rotavirus infection.
- FDA put the vaccine on hold for 8 weeks for additional safety testing of the pig virus

- More than 20 collaborations with academia, government agency and pharmaceutical companies
- Multiple licensing discussions underway.

Contact: Crystal Jaing

Accelerator Mass Spectrometry (AMS) precisely measures rare isotopes in tracing studies

Plasma Profiles of Acetaminophen and major metabolites following an oral microdose

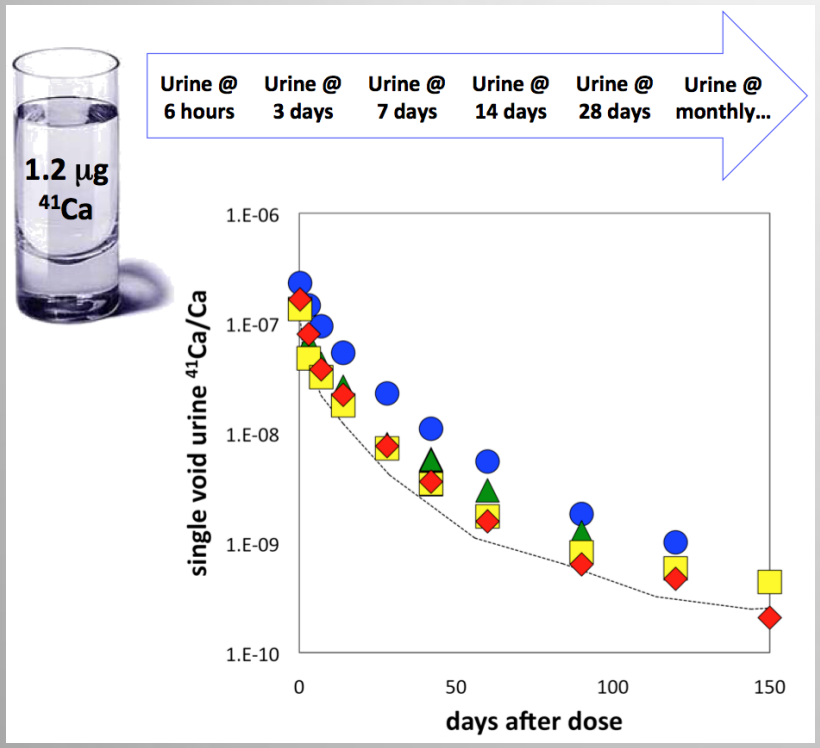


- AMS measures the relative abundance of long lived radioisotopes (e.g. $^{14}C/C$, $^{3}H/H$, $^{41}Ca/Ca$) by counting atoms.
- Used in microdose studies to trace parent and metabolites (ADME)
- Identify targets of test compounds
- Small sample requirement (< 1 mg).
- Detection limits ~ 10^6 atoms - attomole quantitation in mg sample sizes.

Measure physiological concentrations of isotopically-labeled compounds in humans.

Contact: Bruce Buchholz

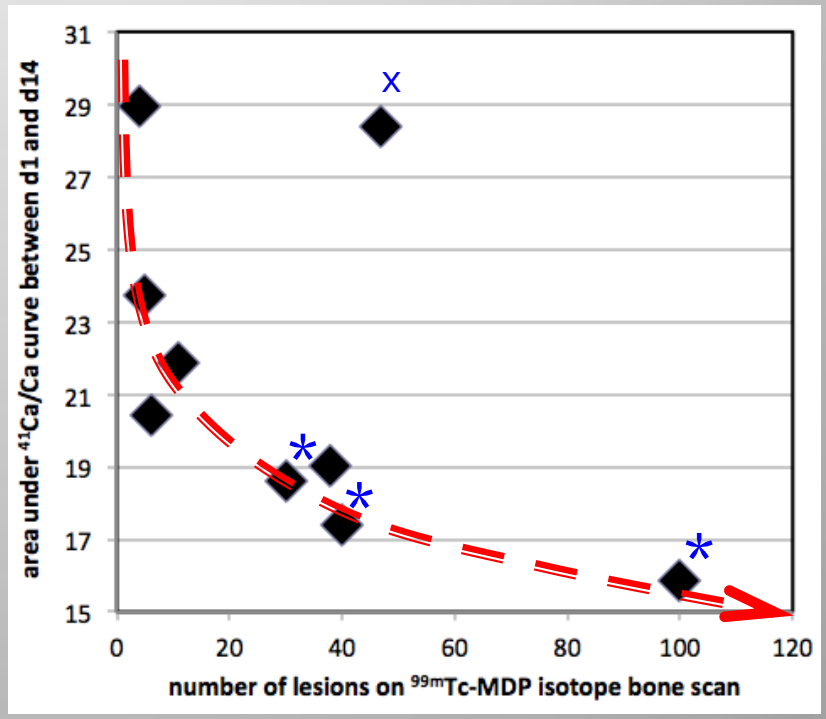
Urine $^{41}Ca/Ca$ stratifies disease extent, risk for complications and shows therapeutic response



One microgram of ^{41}Ca safely and permanently labels the human skeleton.

First two weeks of urine correlate with metastatic extent measured via bone scan.

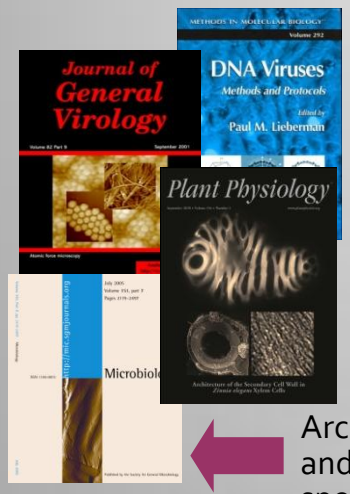
* subjects who died prior to end of 18-month study
x one outlier – unique initial calcium kinetics



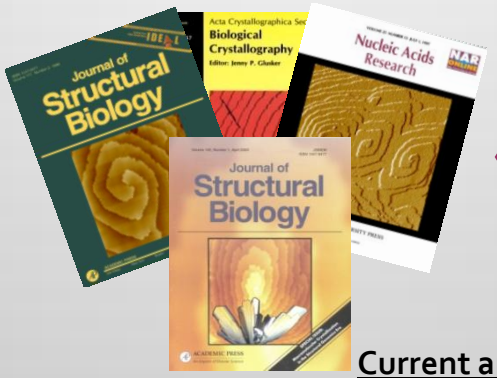
Contact: Darren Hillegonds

Technical Approach: High-resolution biophysical analysis

- **High-resolution biophysical analysis**: Direct insights into molecular architecture and structural variability of biological systems as a function of spatial, temporal, developmental and environmental organizational scales
- **LLNL team** is developing *in vitro* AFM for studies of architecture, physico-chemical properties, and function of macromolecular assemblies, pathogens and cellular systems
- **LLNL strong leadership positions** in AFM studies of architecture and function of macromolecular assemblies and pathogens (7 book chapters, 6 reviews, more than 45 research articles)



Architecture, structural dynamics and function of bacteria, bacterial spores, plant and live mammalian cells



Molecular-scale mechanisms of assembly and crystallization of a wide range of macromolecules

Current approaches:

- Multi-probe (AFM, FTIR, NanoSIMS) physico-chemical nanometer scale characterization of complex biological systems and processes
- Collaboration with leading bio-scientists

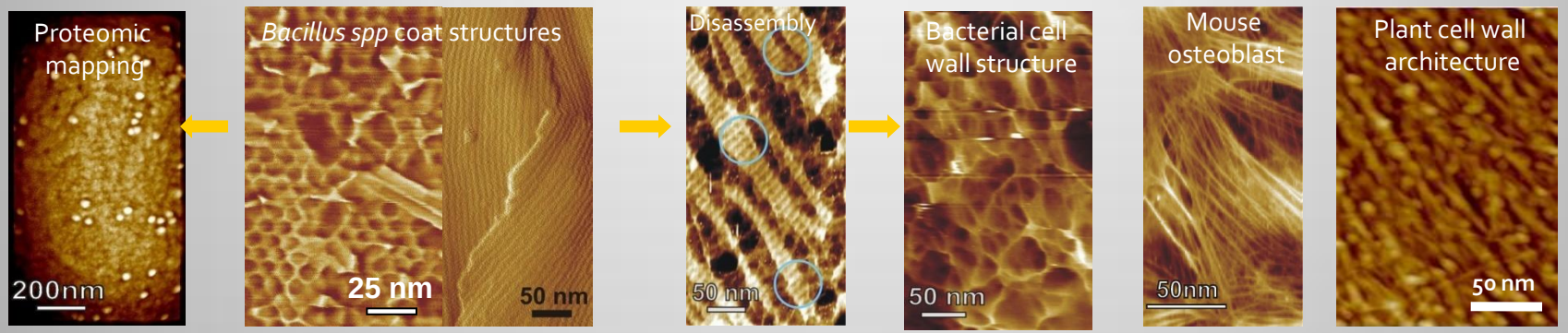
IMPLEMENTATION:

- Identification/Characterization of Species/Processing-specific Biological Structures and Processes
- Proteomic Mapping of Cellular Systems and Pathogens
- Mechanisms of Protein Regulation of Cellular Processes and Pathogen Life Cycle/ Disease
- Bioforensics and attribution

Contact: Alex Malkin

Single Pathogen/Cell Nanoscale Characterization of Microbial/Cellular Architecture, Assembly, and Life Cycle

Selected Publications: M. Plomp, T.J. Leighton, K. A. Wheeler and A.J. Malkin (2005), *Biophys. J.* **88**, 603.
M. Plomp, T.J. Leighton, K. A. Wheeler and A.J. Malkin (2007), *PNAS*, **104**, 9644
C.I. Lacayo, A.J. Malkin, H.-Y. Holman, L. Chen, S.-Y. Ding, M.S. Hwang, M.P. Thelen (2010). Plant. Phys. **154**, 1.
A.J. Malkin (2010). In: Life at the Nanoscale: Atomic force microscopy of life cells. Pan Stanford Publishing



Selected recent research highlights: *First single spore/cell molecular scale characterization of:*

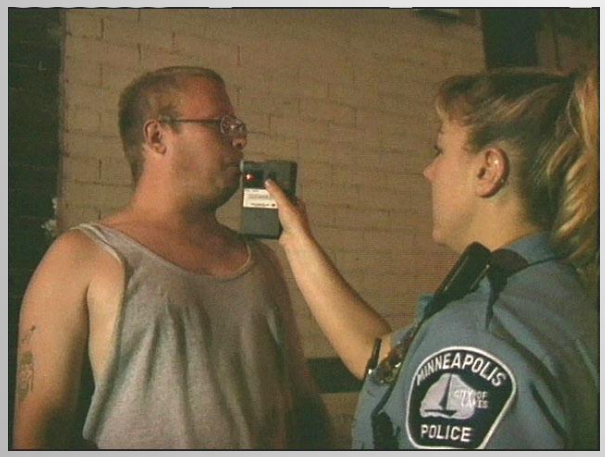
- Species/preparation-specific native structures of *Bacillus and Clostridium* spores
- Proteomic mapping of pathogen, cellular and microbial structures
- Novel bioforensic approaches
- *In vitro* real-time near-molecular structural dynamics of single pathogen during its replication cycle
- Dynamic responses of metal-resistant bacteria to toxic metals
- Structural dynamics of mammalian cells during locomotion, tissue formation, cell division, and cell death

Current and Future Directions:

- Multi-probe high-resolution biophysical and chemical approaches combined with biological tools/ Viral and cellular systems
- Mechanisms of protein regulation of pathogen life cycle/ disease
- Direct probing: Impact of therapeutics, environmental insults, and other stimuli
- Proteomic architecture of live cancer cells and their responses to various stimuli and therapeutics
- Structural dynamics during cellular proliferation and differentiation: Cytoskeleton, adhesion, motility, and apoptosis

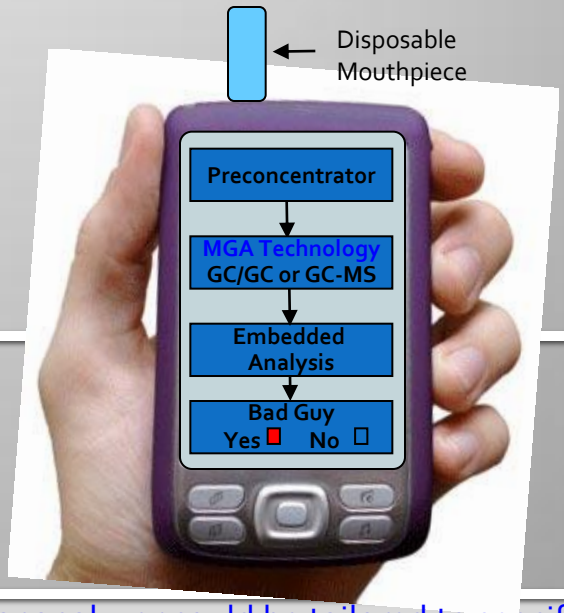
Vision: Real-time breath analysis with miniaturized systems - new capabilities for biomedicine and counter-terrorism

Vision: A handheld device that requires only one breath for automated analysis in seconds; >100,000x more sensitive than alcohol breathalyzer while analyzing multiple trace gas markers.



- Most current breath analysis technology is bulky and slow (~many minutes to hours) and not ready for field applications.
- New miniaturized systems for rapid screening of breath could be developed using technology that is currently under development (e.g. MGA program by DARPA)

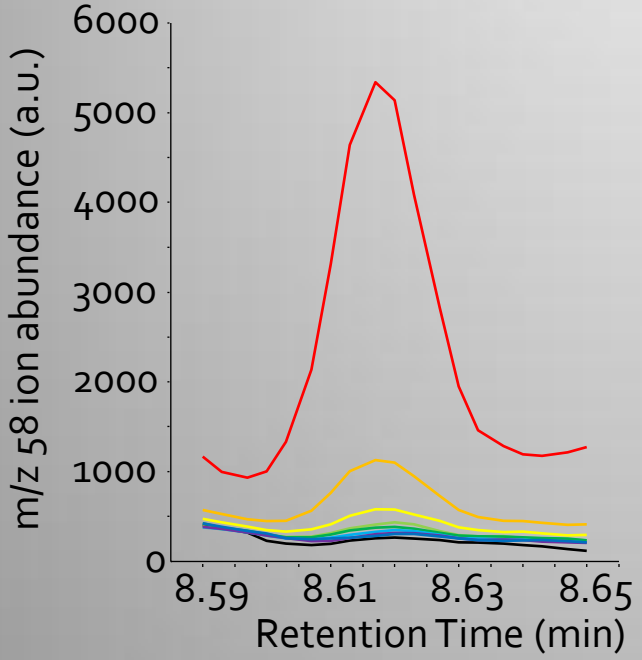
- Biomedical diagnostics using breath analysis very promising: non-invasive, relatively fast
- Some human activities leave long-term traces detectable in a subject's breath
  - Exposure to chemicals, e.g., in drug manufacturing and handling
  - Exposure to CBRN weapons and materials
  - Physiological state and stress of a subject
- More R&D work will be needed to explore and confirm breath signatures in the above areas!



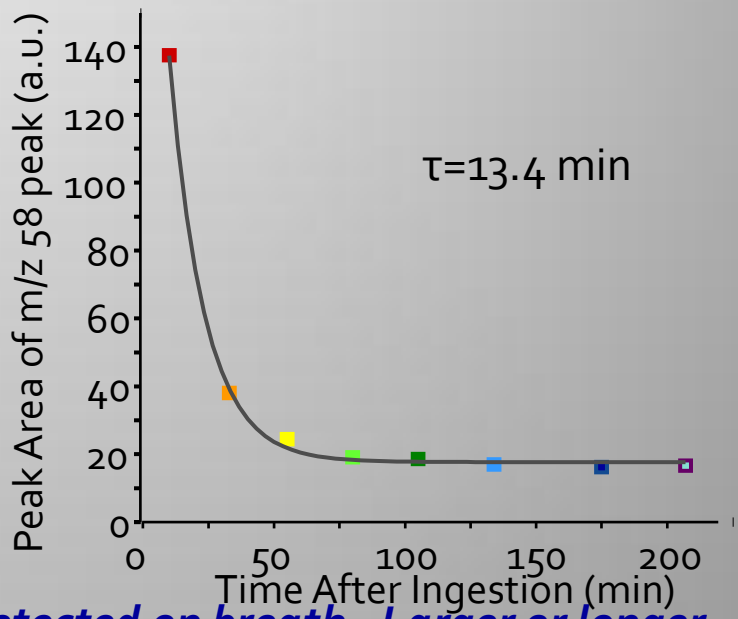
Modular analyzer could be tailored to specific applications

Drug Exposure Detected in Breath

A subject ingested 2 tablets containing 30 mg pseudoephedrine HCl with water. Analysis of the exhaled breath vapor (EBV) collected after this exposure revealed a peak attributed to pseudoephedrine. The area of this peak showed a rapid increase followed by an exponential decay.



11 min after ingestion
11 min after ingestion
33 min after ingestion
55 min after ingestion
80 min after ingestion
105 min after ingestion
134 min after ingestion
175 min after ingestion
207 min after ingestion
Before ingestion

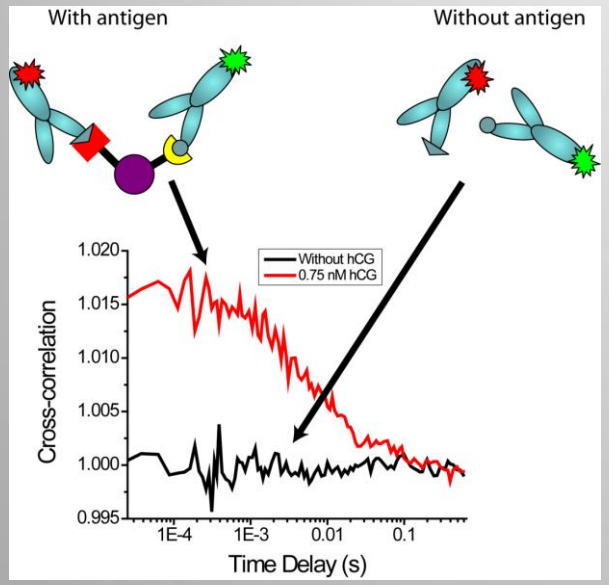


Exposure to a small amount of pseudoephedrine can be detected on breath. Larger or longer exposures would provide even more signal for detection.

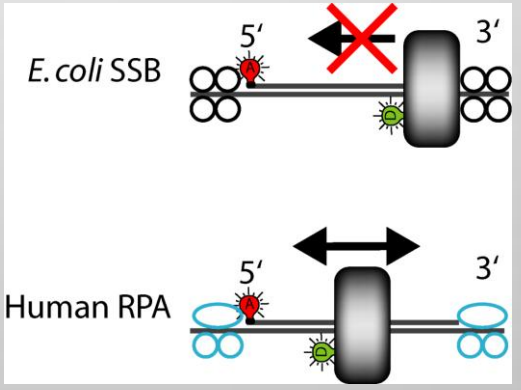
Contact: Matthias Frank

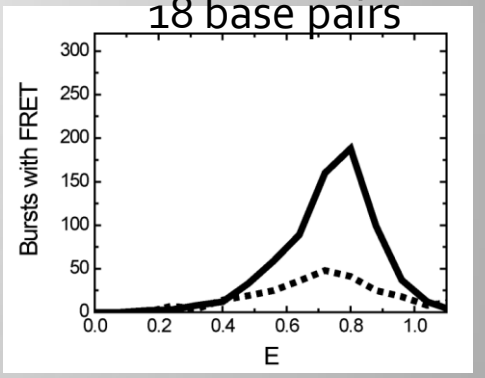
Single molecule fluorescence provides novel observables for diagnostics

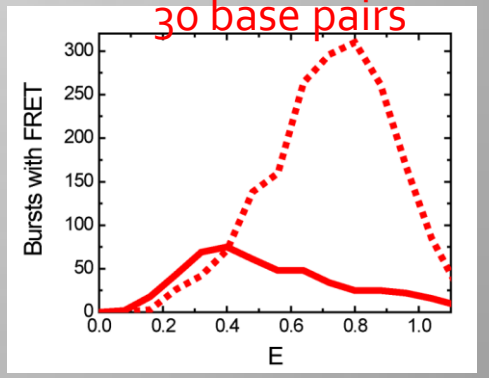
Simple, mix-and-read protein detection



Detected DNA sliding clamp – SSB interaction using single molecule FRET



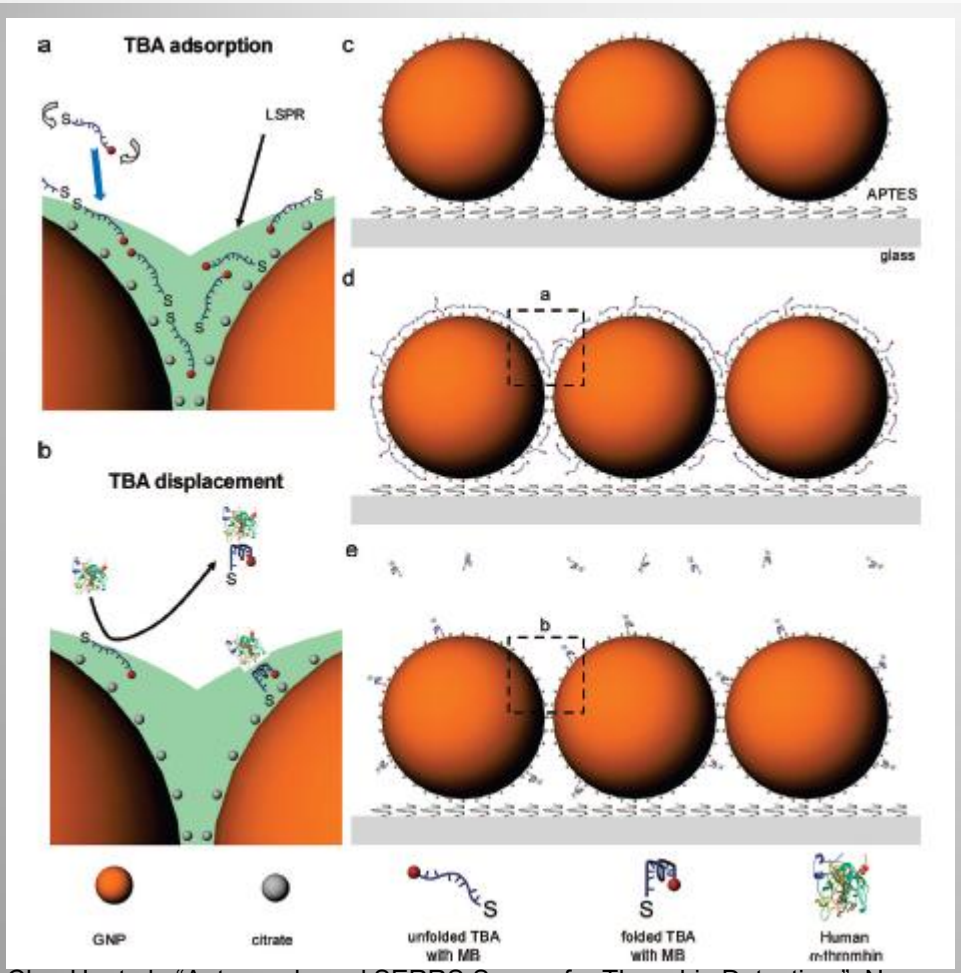




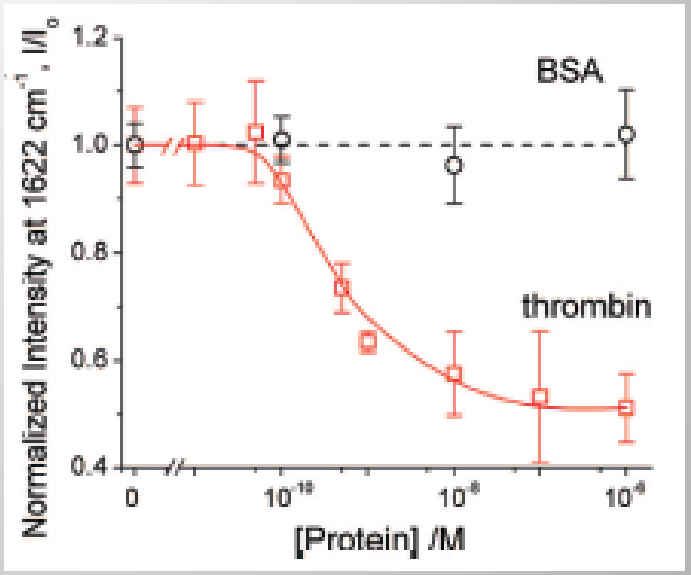
Solid lines: *E. coli* SSB
Dotted lines: Human RPA

Contact: Ted Laurence

Protein detection using DNA aptamers and nanoplasmonics: SERRS or SEF



Cho, H. et al., "Aptamer-based SERRS Sensor for Thrombin Detection." Nano-letters, 2008. 8(12): 4386-4390.



Publication detecting VEGF under review.
Collaboration between CBST at UC Davis (Steve Lane), UC Berkeley (Prof. Luke Lee), and LLNL (Ted Laurence, Brian Baker, and Jane Bearinger)